

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS

1. (*currently amended*) A method of determining the copy number (CN) of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase, probes and optionally, any additional reagents required for amplification;
- (2) performing one or more amplification cycles to amplify the NucSeqI, wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII), and the following amplification steps are carried out:
 - (a) NucSeqI is amplified,
 - (b) NucSeqII is amplified,
 - (c) a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI and present in a control sample is amplified at multiple dilutions, and
 - (d) a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII and present in a control sample is amplified at multiple dilutions,

wherein

- (i) the ratio of concentration of NucSeqI' [[and]] to the concentration of NucSeqII' is known,
 - (ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of [[the]] NucSeqI' and NucSeqII' at multiple dilutions results in the generation of standard curves SC_I and SC_{II}, respectively, at multiple dilutions,
 - (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and
 - (iv) NucSeqI' and NucSeqII' are localized on a single vector; and
- (3) determining from the results of the amplifications of step (2) such that the concentrations of NucSeqI and NucSeqII are determined by using the respective standard curves SC_I and SC_{II}, to obtain the relative such that copy number CN of NucSeqI relative with respect to NucSeqII is determined using by the formula:

$$\text{relative CN} = \frac{\text{Conc-I}_{\text{SCI}}}{\text{Conc-II}_{\text{SCII}}}$$

wherein, in said formula,

- (i) "relative CN" is the ratio of the relative copy number CN of NucSeqI relative to the CN of NucSeqII in the sample;
- (ii) "Conc-I_{SCI}" is the concentration of NucSeqI determined using from standard curve SC_I; and
- (iii) "Conc-II_{SCII}" is the concentration of NucSeqII determined using from standard curve SC_{II}[, and]]

wherein at least one pair of amplification reactions selected from (a) and (b), and (c) and (d) is performed in a single container and monitored spectrophotometrically during amplification, and NucSeqI and NucSeqII are localized on a single vector.

2. (*currently amended*) A method for determining the absolute CN of a nucleotide sequence NucSeqI in a sample, comprising:

- (a) determining the relative CN using the method of according to claim 18, and wherein an absolute copy number is determined by
- (b) multiplying the relative CN by the absolute CN number of copies of NucSeqII per cell.

3. (*previously presented*) A method according to claim 1, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI sequences are localized on a single vector.

4. (*previously presented*) A method according to claim 1 wherein the sequences of NucSeqI and NucSeqI' are the same.

5. (*previously presented*) A method according to claim 1 wherein the sequences of NucSeqII and NucSeqII' are the same.

6. (*previously presented*) A method according to claim 2, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

7. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. *(previously presented)* A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(previously presented)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(previously presented)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(previously presented)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(previously presented)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(previously presented)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(previously presented)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.

17. *(new)* A method according to claim 1, wherein the sample is derived from cells.

18. *(new)* A method according to claim 17, wherein an absolute CN of NucSeqII per cell is known.

19. *(new)* A method according to claim 18, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

20. (new) A method according to claim 18, wherein the sequences of NucSeqI and the NucSeqI' are the same.

21. (new) A method according to claim 18 wherein the sequences of NucSeqII and the NucSeqII' are the same.

22. (new) A method according to claim 19 wherein the sequences of NucSeqII and the NucSeqII' are the same.

23. (new) A method according to claim 20 wherein the sequences of NucSeqII and the NucSeqII' are the same.

24. (new) A method of determining the CN of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase, probes and optionally, any additional reagents required for amplification;
- (2) performing one or more amplification cycles to amplify NucSeqI, wherein the sample comprises a second nucleotide sequence II (NucSeqII), and the following amplification steps are carried out:
 - (a) NucSeqI is amplified,
 - (b) NucSeqII is amplified,
 - (c) a third nucleotide sequence I' (NucSeqI') corresponding to NucSeqI and present in a control sample is amplified at multiple dilutions, and
 - (d) a fourth nucleotide sequence II' (NucSeqII') corresponding to NucSeqII and present in a control sample is amplified at multiple dilutions,

wherein

- (i) the ratio of the concentration of NucSeqI' to the concentration of NucSeqII' is known,
- (ii) standard curves SC_I and SC_{II} comprising at least two reference points are generated by amplification of NucSeqI' and NucSeqII', respectively, at multiple dilutions,
- (iii) at least one pair of amplification reactions (a) and (b) or (c) and (d) is performed in a single container and monitored by fluorescence during amplification, and

- (iv) NucSeqI' and NucSeqII' are localized on a single vector; and
- (3) determining from the results of the amplifications of step (2) the concentrations of NucSeqI and NucSeqII using the respective standard curves SC_I and SC_{II} , to obtain the relative CN of NucSeqI with respect to NucSeqII, by the formula:

$$\text{relative CN} = \frac{\text{Conc-}I_{SC_I}}{\text{Conc-}II_{SC_{II}}}$$

wherein, in said formula,

- (a) "relative CN" is the CN of NucSeqI relative to the CN of NucSeqII in the sample;
- (b) "Conc- I_{SC_I} " is the concentration of NucSeqI determined from standard curve SC_I ; and
- (c) "Conc- $II_{SC_{II}}$ " is the concentration of NucSeqII determined from standard curve SC_{II} .